JEFFREY MARK DRAZEN: So this is how I view pharmacogenomics. It's the use of genetic information to determine who will respond favorably or unfavorably. And I think, actually, unfavorably may be a better phenotype to a given type of treatment. And the reason I say that is that, especially the condition that I study, asthma, there are a lot of folks that have a placebo effect from treatment.

And so if I'm studying a drug and I give everybody a placebo-- that's my trial. I compare placebo A to the pink placebo, to the yellow placebo. And I know this is just a sugar pill and shouldn't have any biological impact on asthma. But I know that 25% of people are going to get better from this. And if I'm stratifying my outcome by genotypes, you'll see that I could be misled.

But if I'm looking for a phenotype which is less likely to be contaminated by a placebo effect-- for example, people who get worse with treatment-- then I'm looking at the natural history of the disease. And I'm less likely to be confounded by that. Now if you're studying something like cancer or heart disease with a hard end point, which is death-- heart disease with a soft end point, which is chest pain-- it's hard to know.

But heart disease with the heart end point, something that's easy to define, you don't have this big a problem. And you can begin to use favorable and unfavorable responses. So I think it's a little more robust phenotype. What it means is that whenever I do a study, I have to think about the contaminating effect of this.

Looking at my data. I know that some of the people whom I think are better because of treatment or, in this case, because of a treatment by genotype interaction are just better by chance, and that there are more likely to be people better by chance in the placebo group than there would be if there was a no treatment group, just because of the nature of that effect. So it's a little bit of a contamination but not a big problem.

So the idea of pharmacogenetics is that there's variability in treatment response. So if I treat a bunch of people-- that some people are going to get better. Some people are going to stay the same. Some people can get a little worse. Since we don't think the medicine makes them worse, usually we think that it's the disease getting worse because the medicine is ineffective.

So the first thing that you want to do if you want to study the pharmacogenetics of anything, you want to look to see how variable the treatment response is among members of a population. And there needs to be substantial variance in treatment response among members of a population. So I'm going to give you an example now, from the asthma world, of a study in which there was variation in the treatment response among members of a population.

So this is a clinical trial. It's easy to understand. Here are patients that are enrolled with mild to moderate asthma. And this is asthma which is more of a nuisance than a real medical problem. So if patients are not treated, it doesn't represent an ethical issue. So that's how we can get away with a two-week placebo run, in theory, because it's OK not to treat people. And are then randomized to receive beclomethasone, which is an inhaled steroid and is one of the standard asthma treatments compared to montelukast, which is an antagonist to the action of the cysteinyl leukotrienes at the CysLT1 receptor.

And the primary outcome in this and a lot of the data I'm going to show you is the forced expiratory volume in one second to 12 weeks. So as an aside by way of pulmonary physiology, I don't think we recognize any of you from suggestive pulmonary class. No, none of you are taking that. If you breathe in all the way to the top and then breathe out as hard and fast as you possibly can-- something like you might go on your birthday.

If you have a lot of candles to blow out-- how fast you can breathe out beyond a certain level of effort is an intrinsic property of your lungs and airways and not how hard you're trying. So you have to try hard enough to reach that plateau. And that is not a superhuman effort. Almost everybody with a little bit of effort can reach that level where how fast they can breathe out is not related to how hard they're trying to breathe.

So that if we measure the amount of air that comes out in one second , we're measuring an intrinsic property in the lungs and not the motivation of the subject. And it's beyond a certain level of effort. And the way you determine whether your subjects are motivated is usually do two or three maneuvers. We have somebody coaching them through it. Deep breath in and out. Blast it out so you get to see whether they're actually trying.

And if this test is done correctly, the variability among tests done in a row is 3% or 4%, even among you all who've never probably done this test before. Maybe one of you have asthma. Statistics would be that one or two of you would have asthma, have done tests like this. Without any coaching or with minimal coaching, can get tested and reproducible within 3% or 4% off the street. So it's a very useful test because it's easy to do.

And we use it as an outcome indicator in a lot of breathing trials. And just to help get you straight. A bigger FEV1, the more air you can get out in one second, the better off are. So that's kind of this outcome indicator. So here are the data. And this is the trial outcome. This is what was published in this journal. And you can see that the patients assigned to the beclomethasone arm improved their FEV1 about 11% to 13% on average. While the people in the montelukast group improve it 7% to 9%.

Now we know from studies done in patients with asthma that if you improve your FEV1 about 10%, that you can perceive it. It's something that you say, hey, I'm better from whatever you just did. Below that, it's hard to distinguish. Above that, if you improve it twice as much, you really don't distinguish it that much, unless you happen to be engaging in really heavy exercise. So it's almost a dichotomous kind of variable, people who got better, people who didn't get better. You'd look at these data and you'd say, gee, it looks like beclomethasone is almost twice as good as montelukast.

So here are the data now shown in histogram format where on the vertical axis, we have the percentage of patients. And on the horizontal axis, we have changes in FEV1. So the patients that will be over on this end had a big response. Patients over here actually got worse with treatment. And the null is right in the middle. And this 10% to 20% bar, anything over this direction, is an improvement. I don't know how to get that to go away. Usually these little boxes go away if you leave them alone long enough. Let's see if I do it again. No, cancel.

So here we are with montelukast. And you see that there were about, altogether, 5% of the population who really did great. There were about 7% of the population that actually got a lot worse. And that it turns out that it's about 42% of the population that improved a significant amount. Now compare this to beclomethasone, which, remember, on average, did twice as well. Well, here's the difference.

There were twice as many people that did spectacularly well. But on the average not a lot. It works out to be 10% versus 5% in some. And rather than having 7% of people who did poorly, it was just 4%. So it turns out that if you trim these by getting-- like they do sometimes in the Olympics. They get rid of your best and your worst scores. If you trim this in that end, the groups are almost identical.

And so the difference in the means reflects poorly on actually what the difference would be in the population. Because if you're a patient sitting in my waiting room and your FEV1 one's improved by 60% and you're sitting next to somebody whose FEV1's improved 0%, the average improvement between you is 30%. But both of you didn't get better, even though the inference would be that you are [INAUDIBLE] better.

So here we see a big population variability in response to montelukast and inhibitor of the actions of leukotrienes and leukotriene receptor in beclomethasone. So I'm going to explore the basis of these two and, depending on time, one more treatment in asthma. And there's big population variability. And the second thing that you need to know-- before I get to this, which I didn't bring you the data to show-- if I study you once, and you're over here, and I'm looking at a genetic event, that is something that you inherited, whenever I study you, you ought to get it better with this medicine.

Because if it's a genetic event, it ought to be something that is imprinted on you and stay that way. While if it's variable from time to time, then it's not going to be useful. So you need to know how repeatable the treatment response is. If the basis of the variance is genetic, it ought to be highly repeatable.

While if it's due to random noise like the reason you improved so much here was that during the two-week running period, you'd spent time with your mother-in-law who has six cats. And you're allergic to cats. So your lung function was low before you started. You then got better during the treatment period because you got away from the cats. If I then restudied you, you wouldn't get this kind of response.

AUDIENCE: [INAUDIBLE]

JEFFREY MARK DRAZEN: Well, so actually I can show you those data, depending on how much time we have. I'll show it to you right now. It's out of the context of this talk. Just pardon me one second. I'll see if I can find-- I have these data actually in another talk. No, no, it won't take too long. Hopefully, it won't take you long to find it. could Let's see. No, it's not that one. I'll give it one more try here. Just look in a different place. I think it's here.

No. One more try. Thought it was there. The nice thing about having a big hard drive, you can carry all sorts of stuff around. See if it's in this one. Yeah, here they are. OK, so here data from a trial. And I was lucky. Because this is a different drug. But this acts like a 5-lipoxygenase pathway. And the drug company that I worked with gave me the data. They sent it to me on a CD.

And a lot of drug companies won't do that because they're afraid that you're going to do something that will hurt them with respect to their market position. They tend to be really paranoid. So to get on this graph, you had to improve your FEV1 by 12%. The consensus definition about what you can perceive varies between 10% and 12%.

So when I did this graph, it was four or five years ago. I drew the 12% line. There were 240 people in the trial, 97 of which improved their FEV1 by 12% on the eighth day of therapy. They were then studied on day 36, 64, and 92. And this graph-- when I graphed the data on my computer, it had all these lines lying on top of each other. And they didn't get any thicker. So I can strain the data through this point here.

So this isn't a data point. But this is simply to allow you to see how many people are in this group that are in that group. It's a graphical representation thing. I mean, if I had clinical data that looked like that, I wouldn't believe it. So anyhow. What you can see is that of this group of people who improved, some actually got worse and stayed worse. A lot of them got better and stayed better. And there were some that kind of moved around.

If you follow the dots carefully, you'll find that three fourths of the people are above this 12% line on two of the three subsequent occasions. So that's active treatment with zileuton, which is the active drug. Here are the same entry criteria, same trial treated with placebo. And you can see that, as I told you, there are placebo responders. These people got better and stayed better.

In this trial, however, of the people who were above on day eight, only about 40% are persistently above the line. And, in fact, you can see there are a bunch of people here who flunked out. And then they kind of went up and down. Now if you look at the people who don't get better. These are nonresponders to treatment, which I defined as 5% or less. So I left myself a zone of middle response.

So there are a bunch of people who don't get better. And, in general, if you don't get better, you stay not better. Although there were a few people who were kind of late bloomers. It's a relatively small proportion. And when you look at placebo, it looks about the same. So you can see why the nonresponse phenotypes a little better than the response phenotype in terms of the noise. Because the people who don't get better and the placebo people who don't get better look pretty much alike.

Well, the active treatment guys are contaminated by these people who you would say were better when, in fact, they weren't. So there you go. That should answer the question there. So I can close this one. I guess I closed the one I was still working on. Here you are. And so we're talking about repeatability. Have you heard about Sewall Wright and how he is a population geneticist? He did most of his work at the University of Chicago. And he died about 15, 20 years ago.

He wrote a four volume series on human population genetics before we had any markers or anything. And he kind of forecast and did a lot of the primary mathematics, which are the basis of population epidemiology. And he actually derived an equation for what he calls repeatability. Now what I would really like to know is the heritability of the asthma treatment response. But to do that, I'd have to study multigenerational families.

And there are three problems with that. One is that asthma treatment is changing. Second is that we know that an asthmatic at age 10, at age 25, and at age 55 responds differently to treatment. Their disease tends to get ingrained and unresponsive to treatment. And so you'd have to be able to study, let's say, all the asthmatics within a family at age 20. But the asthma treatment that you gave the patient's father is no longer available. There's something better.

And so you aren't going to be able to get that treatment. And, of course, the patient's father is now 50. And you can't treat him because the asthma treatment response varies. So it's going to be almost impossible, I think, to get the data you need to determine the heritability of asthma treatment. So we look at the repeatability. And the calculated repeatability for beclomethasone and montelukast from clinical trials. And we've done on the order of 80%, which is good but not awesome. But repeatable enough we think that there's a pharmacogenetic signal. Yeah.

AUDIENCE: I have just a more basic question about what [INAUDIBLE] heritability [INAUDIBLE].

JEFFREY MARK DRAZEN: So now we're not talking about the heritability of the asthmatic treatment response. We're talking about the heritability of asthma. And the asthmatic phenotype is got about-- in twin studies varies but is on the order of 65% or so, comparing monozygotic and dizygotic twins.

AUDIENCE: Is there any reason to connect those two [INAUDIBLE] treatment?

JEFFREY MARK DRAZEN: Well, one of the real problems with asthma is that it's difficult-- it's like an impressionistic picture. If I'm looking at it from 30 feet, it's easy to see what it's about. As I get really close up, I lose the picture. And it's very difficult to define because there's no biochemical test I can use. Quite often people look at the theorem immunoglobulin E levels. Because it's related to allergy but not asthma. But it's a very quantitative phenotype.

And so the definition of asthma is airway obstruction, which varies spontaneously as a result of treatment. The problem is-- you take two people that have the same genetic background, but one of them is exposed to a lot more allergen than another. Even if you have monozygotic twins and one of them plays basketball, and changes his clothes, and wears gym clothes that have been sitting around in a ratty locker, and gets that exposure every day while the other one's a swimmer and doesn't have the same kind of exposure, one of them will develop asthma and one may not.

And so you'll say it's not inheritable when, in fact, it requires both a genetic background and an environmental exposure to manifest the phenotype. The classic examples are there a lot of people from Hong Kong who have latent ragweed allergy. And they're OK in Hong Kong. There's no ragweed or very little in Hong Kong-- move to the States where there's a lot of ragweed. And they get really asthmatic in the fall.

Well, they didn't have a problem back home because there wasn't the allergen. Come here and they have allergen. And they get exposed. So it's a combination of environment and genetics that appears to bring the disease on. The research I'm going to tell you about represents the work of a whole pile of people. This is most for the pile, shown here.

There are people at the Channing lab, which is [? free-- ?] have heard from any of these folks, [INAUDIBLE], I don't know. [? Zach ?] works with some of them. The Pharmacogenomics Research Network, the Asthma Clinical Research Network-- these are both NIH sponsored consortia that I've been part of. And the Whitehead Institute-- I'm sure you've all heard of those folks over at MIT. So I'm going to talk about these three asthma treatments. These are three of the major asthma treatments and what we know about their pharmacogenomics. And we'll see how things go.

So with this first one, we used a candidate gene strategy. You're familiar with candidate gene strategies? So this is work I started about a decade ago. I had a post-doc from Canada who was interested in the problem. And I said, what we want to do is to look at the 5-lipoxygenase pathway. That's the leukotriene pathway. Because we knew the biochemistry of drugs.

And I showed you the data, the red and green lines were from this drugs zileuton that inhibits the action of the enzyme 5-lipoxygenase. In fact, I've been involved in the development of these four drugs worldwide. And so we knew the structure of this enzyme. We knew its genomic sequence. And our question was, when you have patients with asthma who show up in your office, they all have phenotypically similar asthma. But we think that some of them may have asthma because they have an excess of 5-lipoxygenase products and stimulation at this receptor.

But a clinically indistinguishable phenotype can be someone that has asthma due to an excess of substance P, a neuropeptide having nothing to do with this. Or you can have somebody that has excessive of histamine, or someone that has an excess of neurokinin A, or endothelin. So there are probably half a dozen endogenous bronchoconstrictors, which have totally different biochemical pathways that can lead to the same clinical phenotype. And so you recognize that as asthma. And they probably represent people that have a different genetically programmed mast cells in neural responses.

So are argument went that if you could look at the variability of the treatment response here related to the enzymes in this pathway, you'd be able to begin to pick out people whose asthma was associated with leukotrienes and specifically whether there would be variability here. So what we started with is we had the human 5-lipoxygenase gene. We knew it's intron and exonic structure. When I started the work, this was hot, new information.

And this guy from Canada worked on the problem for a month and quit. He said it was too hard. And I was really blessed. I had a post-doctoral fellow from Korea-- actually he was a visiting scientist. And he's probably the most patient person in the entire world, I think. And he plowed his way through this gene, exon by exon, doing old-fashioned sequencing, SSCP. And this took him almost three years. This kind of work now takes three weeks or maybe three days, depending on what kind of genotyping outfit you have.

And what he found, which was quite discouraging, was that there were no common-- that is alleles greater than the frequency 0.15-- DNA sequence variants leading to a modified protein sequence in the entire 5-lipoxygenase gene. You got a question or are you just stretching? Wouldn't want to have a pulmonary embolism over there. So there were, however, variations in the transcription factor binding region, which is just upstream. It's the 5-LO gene promoter.

Now what was known about the gene promoter at that time, which has actually hung in there to be true, is it just the head of the translation start site, there are a series of SP1 and EGR1 binding motifs. In fact, there are five of them in tandem. Then if you search the GenBank, this is the only gene where that occurs. So the sequence GGGCGG is repeated over and over and over again. And that's just right in the core promoter. And that's where the sequence variants were found.

In fact, the sequence in the GenBank has five of these in a row. And we've identified individuals with 3, 4, and 6, and 7, less commonly, we've, in fact, identified people with 2. And so this is a variable nucleotide tandem repeat. People could use it for genotyping. But we showed that it read true. So it's not one that it tends to expand. It's not like it is in Huntington's disease.

If your mother and father have different alleles and are homozygous for them, you're going to be a heterozygous. So we did enough family studies to know that this was a stable VNTR. And then what we did was we took human cells-- these are HeLa cells-- and transected them with the wild type promoter and promoters which has five repeats, and with 3, 4, and 6 repeats. And studied them in a CAT assay, this is a chloramphenicol acetyltransferase assay. So the higher the bar, the greater the promoter activity.

And you can see that all the mutants are less effective at driving gene transcription than the wild type. Now this is one of these moments that you never forget in clinical investigation. I was going over these data with Tucker Collins, who's a professor of pathology at Brigham now. And he looked at these data and says, you know, Drazen, you're absolutely right. But you've got to find something else to do because these variations are too small. No one wants to study a 35% or 40% decrease in CAT transferase activities, too small.

But what I knew from clinical trials was that if I inhibited 5-lipoxygenase 35% or 40%, that I got a very substantial clinical benefit. So the argument that we had in our head was that if patients with variations in the 5-lipoxygenase, the ALOX5 promoter, had downregulated ALOX5 production, but they wouldn't respond to antileukotriene treatment. The idea is that patients with this funny form of the core promoter would have some other cause for their asthma.

Their asthma may be due to the histamines, or to substance P, or some other variant, some other mediator, but that leukotrienes wouldn't be important. Because these are all downregulatory mutations. So I was lucky, at the time, because Abbott Pharmaceuticals had developed the 5-lipoxygenase inhibitors zileuton. And it had two big problems they were trying to overcome. One is that it has to be given to patients four times a day. And that's a pain.

If you give patients the pill to take once a day, they'll do it. Twice a day is harder. Three times a day requires a saint. And four times a day requires God. It's almost impossible to do it. Now I did actually have patients that had pretty bad asthma that took it four times a day. So Abbott developed the compound you could take twice a day. The second problem for them was that about 3% of people that took this drug developed abnormalities in liver function tests.

And although nobody had a liver that checked out on this drug, it required monitoring of the whole population to find the 3% or 4% of people who had an adverse hepatic response. It's a very common problem in drug development. And so they came up with a daughter of zileuton, which wasn't supposed to have this problem. And it was called ABT 761. It acted at the same location. And that was the plan.

So it was the clinical trial that we designed. I actually helped design it. This was not a high-tech clinical trial. We enrolled patients on no treatment. And we assigned them to-- these were asthma patients-- to ABT-761 or a placebo. And since they were patients that we found on no treatment, continuing them on no treatment seemed like it was no problem. Now this was seven or eight years ago. Asthma treatments progressed. It would be harder to do this trial now. Because there are a lot of people who didn't get treatment [? who would. ?]

So what happened was we genotyped everybody at the 5-lipoxygenase locus. And as we expected-- oh, I forgot to tell you one more thing which was that when the trial was set up, Abbott, to save money, said, look, when a third of the patients complete-- you don't enroll everybody in the first day. Usually, it takes a couple of years to enroll-- but when the third of the patients complete their 12 weeks, we're going to compare the incidence of liver function tests in the placebo and ABT-761 group.

And if this drug isn't improving the rate of adverse effects, we're going to stop the trial. Because it's not worth another $40 million to find out something we don't want to know, which was that this drug wasn't in advance. Because they were hoping it would be in advance. And I thought that was a very reasonable approach to life. So we genotyped everybody. And as you might expect, the wild type allele occurred most commonly, made up about 81% of the alleles.

The deletion alleles three and four made up 19%. The addition allele six was so rare-- with one patient with it-- that it really didn't add up to much. So when we stratified the results of FEV1 by genotype, the patients that received active treatment and where the wild type genotype, 55, improved their FEV1 about 17%. Patients on placebo with the 55 genotype improved FEV1 5%.

Now it turned out that there were 14 patients that had active treatment that had no 5 allele. So we called them active XX. And they actually had their FEV1 get worse. And since we didn't stratify the enrollment by genotype, it turned out there was only one patient that had XX genotype on placebo. And that person got better. But that's one patient. It's hard to make any sense out of that.

So the p-value for this change in FEV1 was 10 to the minus fifth. This is unlikely to have happened by chance. But when we went into the trial based on a preliminary analysis of the data set that we never published because we were concerned about bias in ascertaining the patients, we had thought that heterozygosity at this locus would contribute to the phenotype. And it turned out that that's not the case. You had to be homozygous. So that the allele frequency here is 0.19 squared. So it's very small. It's about 3% or 4% of the population.

So from a pharmacogenetic perspective, it's interesting, but it's not economically interesting. On the other hand, if it had been 20% of the population, it would have been interesting. Because if you assume that you could do a genotype for $50 bucks. It's easier to treat the patient and see if they get better than genotype 100 people to find people three people who aren't going to get better. Since there are also probably other reasons that you don't get better with this treatment other than this genotype. So it turned out to be pharmacoeconomically not very interesting.

Now it turned out that some work we did at the same time was that we got eosinophils. And we look for the expression of mRNA by PCR of the ALOX5 gene. This is our control with [INAUDIBLE]. Then we had five patients here with a 55 genotype. In most of them, but not all of them, have relatively high levels of the five well picked up ALOX5 picked up by PCR. But none of the three patients that we did that had no 5 allele had this availability.

And then we looked at the amount of LPC-4, that is the leukotrienes produced by these NFLs. The deletional variance-- there were four patients for that compared to five here. There was a significant difference in terms of lesser amounts of leukotrienes produced. So that suggested that there was variation in this pathway. We've since looked at the cytosolic phospholipase A2, the LPC-4 synthase, the epoxide hydrolase, all the other genes in this pathway. And we haven't found any other variance related to treatment response.

But we did find-- and this is actually interesting confirmatory data. And this is not our work but the work of others. What they reasoned was that if the variance that changed the amount of look leukotriene produced, that you should get the same pharmacogenetic effect if patients are treated with one of these drugs, which rather than inhibiting the enzyme are receptor inhibitors. In fact, I'll tell you that in the trials done with the receptor inhibitors by others, patients with the mutant form of the genotype don't respond to the receptor inhibitor, so confirming our data. It's always heart-warming.

So it suggests that variations in this pathway can contribute to a small fraction of the variance. So I think it's interesting pharmacogenetics. But it never made it pharmacoeconomically because it's a too small proportion of the population. We really have to, I think, affect 15%-- unless you're talking about a serious toxic event. Now, for example, with the gene thiopurine methyltransferase, which is a gene involved in the metabolism of 6-mercaptopurine, which is a drug used to treat leukemia.

Among members of the population, especially people of Scandinavian heritage, about 2% or 3% of those people harbor an allele where they don't metabolize 6-mercaptopurine at the rate they should. And so if you give them the standard dose, they get toxic. And you can actually kill them with the drug. So when you're treating leukemia now with that drug, it's standard to genotype people to make sure they don't have one of these slow-metabolizing alleles.

And they're now you're looking for a life threatening toxicity, which occurs at low frequency. But because the consequences of missing it are so dramatic and so irreversible, it makes sense to screen 1,000 people to find one. In fact, if you screen someone in Asia, you have to screen almost 5,000 people to find one. But yet, in treating leukemia in Asia, they're still doing it, just because it's something that can be prevented if you're going to a higher class treatment center.

Well, if you were screening at the Mayo Clinic where there's a lot of Scandinavian blood, really make sense to do it. But for something like this, where the failure to respond to treatment is a nuisance as opposed to a life-threatening event, it didn't make pharmacoeconomic sense. So my second example is about inhaled steroids. And steroids are different from antileukotrienes because we knew how antileukotrienes work in asthma. I mean, they were developed based on understanding of the biology of asthma.

But inhaled steroids we knew worked based on observation. But steroids have thousands of potential mechanisms of action. We had no idea which one was accurate. So some of this study is useful because if I can find a gene that controls steroid response, maybe that's telling me a gene that's important in the biology of asthma. And then, I could develop a treatment that inhibited just that gene and wouldn't have a lot of the side effects of steroids.

Because one of the big problems with treating asthma with steroids is that if you're a kid, it makes you shorter. If you're an adult, it makes your bones brittle. It causes acne to break out, causes skin to thin. It's got a lot of side effects that aren't terribly good. And to get rid of those side effects would be good until you can get at it. At the same time, with inhaled steroids, just like with antileukotrienes, you saw that there are about 40% of people who are paying for drugs who aren't getting any benefit.

But the drug companies think that's perfectly fine. I don't. Because they're getting the toxic effects, we know. But they're not getting the therapeutic benefit. And it's costing them $50 bucks a month, sometimes more. The new inhaled steroids are actually $60 to $75 bucks a month. So it's an expensive habit. It's a lot like your coffee habit. If you buy a couple of cups of coffee at the Starbucks or even at the place down here, you're spending two 2 and 1/2 bucks a day on coffee. Works out $70 bucks a month. That's what an asthma attack costs.

So here we are showing two more populations. I showed you the purple population with the beclomethasone There are two different inhaled steroids-- different from the ones I showed you-- showing a variation treatment response quite similar to the one that I showed you from that asthma trial. Now, these two populations are ones that we studied. This is actually a drug company study done by Forest Labs of one of their inhaled steroids. And this was one sponsored by the NIH called the Childhood Asthma Management Program or CAMP.

And even though ones kids and ones adults, totally different study designs, you see this same variation in the asthma treatment response to inhaled steroids in three populations, allowing one to believe it's highly likely to be true. So what we did here is we have a different candidate gene strategy. And the 5-lipoxygenase-- we knew the genes in the pathway. And we could identify them based on how the treatment worked. Here, we were guessing how the treatment worked.

So we got a bunch of people around in a room who we thought knew about steroids. And we compiled the list of possible genes in the pathway. We then looked for DNA sequence variance in that pathway. We then look to see if there's a statistical relationship between clinical response and the presence of the sequence variant. And then we determined their functional relevance. And so I don't need to define SNPs, right? You all know about SNPs and haplotypes, right? Talked all about that.

So here's our strategy. We have 32 control and 16 asthmatic cell lines, which we had an infinite amount of DNA from. And we identified variants from sequencing and from a database. We then selected SNPs based on the allele frequency. We designed genotyping assays and did primary genotyping in the adult study-- that was the drug company study-- of cases and controls.

What we were using is cases and controls was comparing people at this end of the graph to people at that end of the graph. So we call these controls and these cases or vice versa. And then we would identify haplotype tag SNPs that had greater than 5% prevalence. And that gave us first [INAUDIBLE] [? statistical ?] associations in the adult population. We then said if this is true, we should be able to replicate it in the kids. And then we said if it's true still, should be able to replicate it in a third population. And then we did some fancy statistics on it.

So here are the three populations we studied. This adult study is a drug company study, eight weeks in duration, people whose lung function as a percent [INAUDIBLE] is 70% to start. They're adults. They're age 40. And they're interesting. Their steroid response is 7% on average. In the CAMP trial, which is our first replicate, they're kids. So their average age is 9. The trial duration was four years not four weeks or four months. And their average improvement FEV1 is 7%.

And then our second replication trial-- was government sponsored, six weeks. Again, average improvements about 7%. So this is about what you get in these populations. This kind of population, you get about 7% improvement FEV1. This is the list of genes that we interrogated. And this was simply a list of genes made up by understanding the pathway, thinking how it could impinge on asthma. So it's entirely theoretical.

And so here our primary outcome using the single SNP analysis-- these are haplotype tag SNPs. So in the adult study, RS-242941, so this is just the name of one of these haplotype tag SNPs-- using the eight-week FEV1 percent change as a continuous variable, gave us a p-value of 0.025. This is adjusted for covariates but not for the number of [? looks. ?] We then did it in a CAMP population, now many fewer looks, again eight-week percent change, continuous variable, p-value of 0.006. So you're on a roll.

And we looked at that same genotype in the ACRN population and didn't find a relationship. That would have been hitting gold. So we got silver or some other load. I'm not quite sure what you want to call it. But we found that these are three other haplotype tag SNPs. That this one, RS-1876828, which is in the same gene, CRHR1, cortical troponin releasing hormone receptor 1, was positive in both populations.

So we got either second or third prize, depending on your perspective. The ideal would have been to have the same haplotype tags SNP positive in all three populations. What we found was different haplotype tags SNPs in the same gene, suggesting that it's the gene that's the problem but not localizing the site, not actually getting to what we think is the active problem.

So the half-full way of looking at these data are that, gee, although I don't know the mechanism, if I genotype people at these loci, can I predict their response in lung function? So this is the RS-242941. That was the top one in the kids CAMP and the adults. And what we've not done is taken this haplotype tag SNP and looked at people who were homozygous for it, and heterozygous, and didn't have any copies of it.

And you can see that if you own this phenotype, that your improvement in FEV1 is almost twice as great as if you own this genotype on average. When you look at a different haplotype. This is GAT haplotype pair. And this is now in the adult and the CAMP study. Even it's a little more informative here. I can show you the difference in the size of these bars. Here, this is about a 10% to 15%. If you look here, this is now 15% to 20%. This is a little bigger response in people with this haplotype compared to people without it.

So we think that it has some predictive value. It did not turn out to be statistically significant, as I said again, in the ACRN population, smaller number of people. It was in the right sign but not statistically significant. This is a different genotype in that same gene but a different SNP, the RS-1876828, showing again that if you're homozygous AA versus GG, a big difference in response. But when we went back and did this population with that genotype, it didn't work out.

The second thing we learned is that CRHR1 is probably involved in the of the asthma treatment response, suggesting that if you could target CRHR1 antagonist to the lung, where CRHR1 receptor. It's a seven-transmembrane spanning G protein coupled receptor. It's got a bunch of ligands in addition to corticotropin releasing hormone that can activate it.

So some of the questions are, can you find it in the lung? So this is one of these body blots that you buy from one of those companies out west. [? Say, ?] oh, [? it's ?] RNA, if you do, what we did here is 12 cycles of PCR. And you pick it up in the positive control and in the brain. This is a hypothalamic hormone. So the fact that you find the hypothalamic hormone receptor in the brain is no surprise.

If you do twice as much PCR, you not only pick it up in the brain. It shows up in the lung, in the placenta, the thymus, and the lymph nodes. It shows up in epithelial cells and CD4 positive lymphocytes, all tissues that have been implicated in the biology of asthma. So at least it's where it needs to be. Doesn't quite meet Koch's postulates yet. But we're headed in the right direction.

For the third example, what I think I'll talk about is a beta-agonist receptor. So what I've done so far is talk about two association studies. Clinical trials that were done. We then get the data and the DNA and we do a bunch of genotyping and associations but it's all post hoc. So now the story of beta agonists are slightly different. Beta-agonist inhalers are the most commonly used asthma treatment in the world.

I don't know how many of you read the *New England Journal of Medicine.* Have ever see the *New England Journal of Medicine?* Nice graphics. [INAUDIBLE] lollipops in this picture. She's one of the artists that draws them. And so that these inhalers are going off worldwide at the rate of about 1,000 times a second. So there's a lot of-- treat people using this kind of treatment.

So in 1990, almost 15 years ago, this fire was started. A long-acting beta agonist came out. Now the problem with those little inhalers-- they last three or four hours. So this drug company designed a drug that can last and agonise at the receptor for 12 hours. And the hypothesis was that if you took it on a regular basis, your asthma would improve because you'd be chronically dilated. These drugs work by relaxing airway smooth muscle constricted in asthma.

So in this trial designed by the sponsors, they were comparing this long-acting beta-agonist with placebo. And it was a crossover design. So half the patients started on formoterol, half started on placebo for 26 weeks of treatment. They got a month off. And then they were switch to the other treatment and followed for another 26 weeks. And in this trial, the primary outcome was an asthma exacerbation.

Rather than measuring lung function, they said I'm going to wait for your asthma get bad enough for you to be upset by it. And I'm going to click off the asthma exacerbation box when that happens. In the hypothesis had been that during your final treatment, you'd have fewer asthma exacerbations than during your placebo treatment period. Because of the crossover design, everybody's in both arms.

So when these data were published, the asthma world was surprised. Because this is the number of subjects without exacerbation with time. If treatment was 100% effective, your line would go across here. Every time a patient has an asthma exacerbation, the line kicks down. And the patient's then censored from further analysis. And you could see that there for about a week or so they were the same.

But then the patients that got the regular treatment-- remember, the onset prevention was worth a pound of cure. This is supposed to make you better but actually having more asthma exacerbations at any given point in time than the people who only used beta-agonist when they needed it. And when these data came out, there was concern that actually this very commonly used asthma treatment was causing harm. And the fires were fueled by this paper that was published by my predecessors at the New England Journal. I had nothing to do with this, let you know. showing that the use of beta-agonists was associated with the risk of death or near death through asthma.

And what they did in this study is they went to this huge database, which is where every time you get a prescription in the province of Saskatchewan, somebody knows who you are and what prescription you want. And then they also matched that with the asthma exacerbations, asthma death file when people got really bad asthma. Because they have hospital records. And they discovered that the patients that used a lot of beta-agonist either died from their asthma or were admitted to the hospital with very severe asthma. And they concluded it was cause and effect. And they were totally wrong.

This is a classic example of confounding by severity. Or more simply put, the sicker patients were using their beta inhalers a lot. And they're the ones that are more likely to die from asthma. So it was bad epidemiology. But while we were working that out, this trial was conceived by the people at the NIH, who became concerned that a common asthma medicine was making you sick. And so we put together this trial called the beta-agonist study.

All clinical trials have to have a name. Otherwise, you would have to say, remember that trial where they compared drug X to drug Y in a 24-week crossover design trial. It was published in *The Lancet.* remember the bags trial or something like that. So all good clinical trials have a name. This was the bags trial. And in this trial we enrolled patients with really mild asthma for a six-week run-in period when they were on their standard treatment.

And then they were randomized to receive either albuterol, which is a short-acting beta inhaler. So you take it four times a day. This is the one that's used so often around the world. And they were given a coated inhaler. It was white. And it said, study drug. Take two puffs four times a day. And I've already told you about four times a day medication. So this inhaler had in it a computer chip. And the patients knew this. Every time they pressed the inhaler, it recorded the time of day and the date.

And we knew that we had about 95% compliance, at least with pressing the inhaler. There wasn't a video camera in the computer chip. I don't know whether the patient actually inhaled when they pressed the inhaler. But we think that pressing the inhaler meant that they actually used it. And that was a coating inhaler. And they were also given an open-label albuterol. And said, if your asthma is still acting up, you can take this. And so all the patients were given that.

The placebo group was given an identical inhaler, identical instructions, identical computer chip, and open-label albuterol. And the hypothesis was that if regular use of beta-agonist was bad for you, that the blue group would do poorly compared to the green group. Now rather than powering the study for asthma exacerbations, because in these very mild asthma patients, you were taking 1,000 patients studied for a year. We used a surrogate endpoint, which was lung function, strongly related to how patients do with asthma, taken over 16 weeks.

And here the data which we published actually in the New England Journal of Medicine in '96. It showed that the morning peak, which is our primary outcome, the two groups were not statistically distinguishable. Here, it's a very expanded scale. And the difference between these two and 15 liters per minute is probably not significant. We had gone in saying that this difference, 25, was significant. And it got only halfway there.

But interestingly, the blue group did a little poorer than the green group, even though the difference wasn't statistically significant. This wasn't a sign of these treatments being worse for you. But we concluded that they weren't worse for you. In fact, we looked at a bunch of outcomes. Now while we were doing this trial, while data accrual was going on-- this is the beta-2 adrenergic receptor-- a number of polymorphisms were identified in it.

Now these two, which are right up here near the n-terminal part of the receptor, have very high allele frequencies. The minor allele frequencies are on the order of 0.4, and they're very common. And we knew from the work of others that these were functional. That is that an [? RH16 ?] receptor behaved differently than a [? glycine ?] receptor. We also knew that RH16 was in very strong linkage disequilibrium with [? Glyn ?] 27. So that if you were RH16, you were likely Glenn 27.

So what we did was we stratified the data from that clinical trial based on these genotypes, again, a retrospective analysis. And here are the findings. If you had the Gly/Gly genotype and used beta-agonist on a regular basis, your morning peak flow after treatment oscillates around the zero line. If you have the Arg/Arg genotype and only used beta-agonist when you needed it, which works out to be about a puff every other day, nothing happened.

But if you have the Arg/Arg genotype and are using two puffs four times a day, there's a fall in peak flow during and after the active treatment period, suggesting that it's not that the drugs don't work anymore. In fact, when you take them you get bronchodilation. But there's a side effect. When the drug wears off, you're worse than you were before you started taking it. And that's what this is showing us.

Because this is the morning peak flow before treatment. So this says that using a drug on a regular basis makes you worse in the morning than you would be if you hadn't used the drug. But it's genotype related. So that led us to do a perspective trial. And so this is like the gold standard in this business. Rather than sifting through all data, you start off with new data that were designed by BARG trial, beta-agonist receptor by genotype.

And this was two identical trials in which patients were enrolled with the Arg/Arg genotype. A matching Gly/Gly genotype patient was found, at least with respect to sex and lung function. And then both groups of patients were put in this design. And the idea was that in the Arg/Arg patients, we were expecting that placebo would be superior to albuterol. Because we think that these are the patients where albuterol given regularly has a detrimental effect. While in the Gly/Gly patients, this difference would not exist.

And then if we then did a genotype by treatment interaction-- we would say that if you look at Gly/Gly on active treatment versus Arg/Arg on active treatment, that these patients would be better, these patients would be worse, and there'd be a big difference between the two groups. And that's how we designed it. Our primary outcome variable, again, was a measurement of lung function, morning peak flow, with these secondary outcomes.

So we enrolled 332 patients with that genotype. 78 met the criteria. And so this is an interesting trial. Because as far as I know, it's the first trial where people were enrolled by genotype in a non-malignant condition. They came in and they met clinical and physiological criteria. They say, you're a patient for our trial. Now let's genotype you. Because we knew that one out of six people was in Arg/Arg.

If you were then met the criteria and were in Arg/Arg, you were enrolled in the trial. We then went through the people who we determined to be Gly/Gly and looked for a match, sex, lung function, and center where they were studying. So we ended up with these people. These were the baseline characteristics of the two groups. We matched on sex. And we came as close as you could.

In terms of the proportions that were Caucasian, they worked out to be about the same. Their ages worked out to be the same. Their lung functions worked out to be the same. So we had two reasonably matched groups of people that differed with respect to their genotype at the beta-2 adrenergic receptor. So this was unexpected. What happened in this trial was different from our other trials.

We had the patients on beta agonist on a regularly schedule basis. But they had a beta-agonist inhaler they were using if their asthma acted up. So when we did this trial, we switched their rescue inhaler from a beta-agonist inhaler to another type of rescue inhaler called Atrovent, an ipratropium bromide, which doesn't work at this receptor. And we allowed six weeks for patients to get used to using that treatment.

Well, we hadn't expected this to happen. But what happened was in the Gly/Gly patients, their peak flow stayed fixed. Well, in the Arg/Arg patients who were using their albuterol four or five times a day, just kind of in regular use, when we switched them to ipratropium bromide over six weeks, it improved their lung function 30 liters a minute, actually 28 liters a minute.

Now that's as good as you get out of most treatments you pay money for it. And all we did was switch them from albuterol to ipratropium bromide. But in our trial design, we said the zero point is going to be six weeks. So all our comparisons are made from here, where we had this big effect during the run-in, which we hadn't counted on.

Then during the trial-- so the Arg/Arg is yellow. The Gly/Gly is blue-- They start off with peak flows of around 470. The Arg/Args get worse on active treatment while the Gly/Glys get better. While on placebo, the Arg/Args get better and the Gly/Glys get worse. This is the primary outcome peak flow. When we express it this way-- this is now morning peak flow.

And so each of these bars represents a difference between active and placebo. So if active is worse than placebo, you go down. And if active is better than placebo, you go up. Arg is red. Gly is green. And you see that the difference between the two, highly statistically significant, with a value that's on the order of 23 liters per minute. We said 25 would have been clinically significant. It came close but didn't hit that bar.

Then at evening peak flow, the difference is also in the same sign. But interestingly, there have been some recovery of lung function during the day. And our other outcomes. FEV1-- and difference again in the same sign. Arg/Args get worse. Gly/Glys get better. The difference is about 150 liters per minute.

Everybody agrees this is statistically significant. When we look at symptoms. And here, a bigger number is more symptoms. There are more symptoms when the patients are using the drug on a regular basis than they are when they're using placebo. Again, in a sign of Gly/Glys doing better on active and Arg/Args doing worse on active treatment, so highly likely not to have occurred by chance.

Now my final outcome here is how often the patients had to use a rescue inhaler. The ipratropium bromide was the non-beta-agonist rescue inhaler. Albuterol is a beta-agonist rescue inhaler. And we found the same thing. Remember, during this period, the patients are getting albuterol eight puffs a day already. And they're taking more of it. It's not enough. When the Gly/Glys are using at eight puffs a day, they cut back on their daily use. So the difference between these groups-- three puffs a day is highly significant. And in the sign that we're making the Arg/Arg patients worse with active treatment.

So I think the clue out of this one is that about 1/6 out of patients with asthma in the US are probably being made worse by their albuterol treatment, which is actually a very commonly used asthma therapy. Now this study was not calling for asthma exacerbations. To do that, we would have had to study, as I said, about 1,000 people for a year. And to study 1,000 people, you would've had to screen 3,000 or maybe more like 4,000, because it's going to be genotype stratified.

But these two things track. And those kinds of trials are under consideration but are not completed yet. So I've given you three examples-- antileukotrienes where we've shown an effect based on what we know about the biology of the pathway but one of little pharmacoeconomic consequences, inhaled steroids where we weren't quite able to replicate finding [? three ?] genes. Well, we have a gene but probably not a SNP related to the steroid response. It'd also useful to try to do a controlled perspective trial that way.

And then with the beta agonist, we've done the controlled perspective trial showing the genotype makes a difference in treatment response. So that it's more than just an idea, it's something we can actually reduce to practice. And so these are three examples in asthma treatment which tested the advantage of being a recurrent disease requiring chronic therapy. And if I were doing a trial where I wasn't allowed to take patients off treatment, right where the outcomes are going to be strokes, or heart, attacks or terrible events, then you'd be-- not have this kind of freedom.

Even when you're using blood pressure as an outcome. You don't want to let someone's blood pressure go uncontrolled. But here, you can really show a genotype by treatment interaction different kinds of ways. And you can expand this to other kinds of diseases, doing the right kinds of treatment trials where you begin to enroll people by genotype and informative genotypes, which you find out from the evaluation of all data to decide whether this makes sense to move forward.

So this is where I see, to me, one of the major advantages of genetics in actual medicine years. I mean, I can find the genes that cause things. But all I can do is give you bad or good news. You don't have a gene for Huntington's disease. You don't have the gene for Parkinson's disease or you do. Now you're going to worry about it, but you can't do anything about it. I might be able to tell you, you have a gene for a form of emphysema. And you shouldn't smoke.

But still, there's very little the information that I can do with the information about a gene that I harbor or don't harbor, other than making decisions about my kids, and when I want to have kids, and who I want to marry. And it's interesting, in some regions of New York City where they have these arranged Ashkenazi Jewish marriages, they have a number of genetic diseases. They're actually genotyping the people in the arranged marriages so that they don't end up with Tay-Sachs disease and other commonly inherited-- but they're arranged marriages. A lot of marriages these days aren't arranged.

So it's not terribly useful information. If on the other hand you have a disease, and I can look at your genotype and modify my treatment so that in one case, you're likely to get better. In another case, I know that a drug isn't going to work for you. That's a much more medically useful thing to know. I'm sorry that you inherited these genes that are going to cause you to have this bad disease. But at least I know from your genetic profile what treatment you're more likely to respond to it. And so that's why I think-- what is going to make a difference in genetics and medicine? This is going to make a difference.

And we're seeing it now with leukemias. We're going through and doing these studies looking at patients that have a certain kind of leukemia who are likely to have a good treatment response and who aren't likely to have a good treatment response. What that means is that if you're sure in the good treatment response group, I can treat your leukemia and maybe not come so close to killing them.

While if you're in a bad treatment response group, I'm going to get out everything I possibly can from day zero. And I'm going to try to wipe out every cell. So that I think this is much more medically useful than finding causative genes. We have to find genes that help us modify and understand our treatment response. So that's my story.